CONTROLLED REDUCTION OF WATER ACTIVITY IN CELERY: THE EFFECT ON MEMBRANE INTEGRITY AND BIOPHYSICAL PROPERTIES

BY

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Major Department: Food Science and Human Nutrition

Many fruits and vegetables lose desirable texture as a result of dehydration. The mechanisms of textural changes are not well understood. In this study celery was dehydrated to various water activities (a_w) of 0.920-1.000 in order to determine a range of a_w in which various textural and ultrastructural changes occur.

Fresh celery cross-sections were divided into two groups and pretreated prior to osmotic dehydration. One group was pretreated in 20% ethanol (known to impair membrane integrity) and the other in distilled deionized water (DDH_2O). The cross-sections were equilibrated in one of eight polyethylene glycol (PEG) solutions, then rehydrated in DDH_2O. Samples of each of the bathing solutions were taken for mineral, conductivity, and amino acid analysis. Leakage of these components was used as an indicator of membrane integrity. Following rehydration, the celery pieces were blotted and accurately weighed in preparation for physical evaluation which consisted of measurement of rigidity and piece height and observation of ultrastructure.

The ethanol pretreatment resulted in 2-6 times greater leakage than the DDH_2O pretreatment. Electron microscopy revealed extensive membrane damage in ethanol-pretreated samples and very little damage in DDH_2O-pretreated samples. The cell walls and middle lamellae of pretreated samples were indistinguishable from controls not pretreated.

Celery pieces dehydrated to a_w's of 0.995-1.000 were very similar in properties to pieces maintained in DDH_2O. However, celery pieces dehydrated to 0.987 a_w exhibited properties very similar to ethanol-pretreated pieces. Celery pieces dehydrated to a_w's of 0.987 and below had 20% reduced heights, were unable to totally rehydrate, leached from 4-1000 times greater quantities than the undehydrated tissue. They also exhibited rigidities and ultrastructural characteristics similar to ethanol-pretreated pieces. The cell walls and middle lamellae were intact in all tissues of the high and intermediate a_w's (0.920-1.000). The similarity between the properties of the ethanol-pretreated celery pieces and the dehydrated-rehydrated pieces suggests that loss of membrane integrity was responsible for the observed dehydration damage.

The results of this study are discussed in terms of previous studies in the area. Mechanisms of textural changes are proposed and suggestions for future work are presented

INTRODUCTION

Justification

The Institute of Food and Agricultural Sciences (IFAS) at the University of Florida is committed to the development of improved food preservation processes which increase product stability and market flexibility while still maintaining a high degree of quality (Anon., 1983a). Postharvest losses of 20% or $172 million annually of vegetables and ground fruits in Florida are partially attributable to the lack of such processing. The growing popularity of dehydrated fruits and vegetables in the institutional market in the U.S. and the consumer market in Europe makes dehydration an increasingly attractive possibility for fruit and vegetable preservation. In addition, many alternative energy sources are suitable for dehydration and offer possible savings in energy costs.

Although dehydration of vegetables offers many shipping and storage advantages over fresh produce, the rehydrated product does not exhibit the crispness and crunchiness associated with the fresh vegetable. For this reason most dehydrated vegetables are utilized as ingredients in dry soups, spices, stuffing mixes, etc. in which fresh structure and texture are not required (Meier, 1982). The firm, crisp texture associated with fresh vegetables is a result of turgidity. The integrity of the cell wall, middle lamella and plasma membrane is essential for turgidity (Reeve, 1970). Since firmness and crispness are compromised in the rehydrated product, the cell wall, middle lamella and plasma membrane are suspected sites of irreversible damage during water removal. The nature and conditions of possible damage to the cell wall and/or cell membrane have not been well defined.

The following work evaluates ultrastructural changes of these suspected sites during water removal as well as the biophysical differences between fresh tissue and tissue dehydrated to various known water activities (a_w's). Celery was chosen as a model system because of the vast difference in texture between fresh and rehydrated celery. This study is fundamental to the development of processing techniques which may allow for recovery of fresh-like texture in dehydrated vegetables upon rehydration.

Objectives

This study was undertaken to determine the effect of controlled lowering of water activity in cellular components including cell wall, plasma membrane and middle lamella and the correlation of ultrastructural changes to textural parameters. Another closely related objective was to determine a critical water activity below which celery could not be equilibrated and regain texture characteristic of the fresh product.

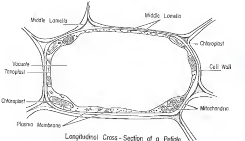
LITERATURE REVIEW

Structure of a Generalized Plant Cell

Figure 1 illustrates a typical celery parenchyma cell. Although plant cell ultrastructure varies according to origin and function, many generalizations can be made. A plant cell is surrounded by a rigid cell wall made up of cellulose microfibrils, which using electron microscopy have been estimated to be 4.5x8.5 nm in cross-sectional surface area (Darvill et al., 1980). The microfibrils are deposited in an ordered fashion outside the plasma membrane (Vian, 1982). A model of a complex three-dimensional matrix of cellulose microfibrils, pectins and hemicelluloses with small amounts of structural protein was developed for the primary wall by Baur et al. (1973), Keegstra et al. (1973) and Talmadge et al. (1973) using sycamore cells in culture. With some variation the model is expected to apply to other angiosperms (Darvill et al., 1980).

The middle lamella, which is mainly composed of hydrated pectic polysaccharides (Hall, 1976), cements cell walls of adjacent cells. Inside the cell wall of living plant cells is a plasma membrane or plasmalemma. Because the plasma membrane possesses semipermeable properties, it serves as a chemical and electrical barrier for the cell. When a cell is turgid, the plasma membrane is closely appressed to the cell wall. Partly because of the difficulty of purifying plant

Figure 1. Longitudinal cross-section of a petiole parenchyma cell in celery.



Longitudinal Cross-Section of a Petiole Parenchyma Cell in Celery plasma membranes, comparatively little is known about them. The fluid mosaic model of biological membranes (Singer and Nicholson, 1972) is the model which best fits the information available (Figure 2). Membranes may be viewed as a fluid liquid-crystalline bilayer of phospholipids with peripheral and integral proteins incorporated in mosaic fashion.

The cytosol, or cellular solution, lies within the plasma membrane. The majority of metabolism takes place here or within organelles in the cytosol. Typical plant organelles are mitochondria, chloroplasts, nuclei, dictyosomes, endoplasmic reticulum, ribosomes and others (Newcomb, 1980). Closely associated with the plasmalemma are microtubules. A variety of functions have been hypothesized for microtubules, ranging from involvement in cell wall synthesis to chromosome movement (Vian, 1982, Inoue, 1981).

Plant cells may also contain one or more vacuoles, which maintain turgor and serve as reservoirs of water and solutes. Mature storage parenchyma cells have large vacuoles and very little cytosol. The vacuole has a membrane, the tonoplast, which actively maintains a separation of the vacuolar contents, mainly acids, sugars, minerals and water, from the cytosol.

Anatomy of Celery (Apium Graveolens) Petioles

Esau (1936) described the main morphological and anatomical features of celery petioles. Celery petioles are crescent-shaped in transverse section. Figure 3 illustrates the major cell types seen in transverse cross-section. Parenchyma cells constitute the major cell

Figure 2. Diagram of the fluid-mosaic model structure from Singer and Nicholson (1972). Circles represent the head groups and two lines are used to represent the hydrophobic tails. Protein is represented by the cross-hatched particles embedded in the bilayer.

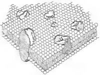
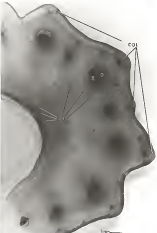


Figure 3. Transverse cross-section of celery petiole. COL = collenchyma bundles, p = phloem, X = xylem, P = parenchyma, OD = oil ducts, and e = epidermis.


COL
1mm

type of the petiole. They are somewhat circular in cross-section and the length is 1.5 to 2.5 times the diameter. Oil ducts dot the parenchyma but are mainly associated with the ribs and vascular tissue. The ribs of the celery on the abaxial side under the epidermis are made of thick-walled collenchyma cells. Each of these is opposite a vascular bundle. The vascular bundle is composed of a bundle cap of collenchyma, xylem parenchyma, phloem vessels and phloem parenchyma. The collenchyma and vascular bundles make up what are colloquially called celery strings.

Esau (1936) evaluated mechanical strength of collenchyma, vascular bundles, the bundle cap and the xylem. By far the greatest strength was exhibited by the collenchyma. Because of the strength of those tissues, it is tempting to view the collenchyma as steel girders supporting a building. In fact, collenchyma may have quite a different role. Esau noticed that strings removed from the Utah variety were coiled as if they had been under pressure. She quoted Ambronn (1881) who measured tensions of 8 to 12 atmospheres on the collenchyma from the turgor of the surrounding parenchyma tissue. The thick-walled cells may be associated with the vascular bundle to protect them from collapse under surrounding turgor pressure and prevent desiccation during stress. Celery petioles are maintained erect largely as a result of turgor pressure (Sterling, 1963).

Structure and Texture of Plant Foods

Differences in fruit and vegetable texture are a manifestation of the ultrastructure and chemical composition of the plant tissue. A number of studies have correlated structure and texture (Jewell, 1972, Lee et al., 1969, Mirza and Jewell, 1976, Mohsenin, 1970, Reeve, 1943, 1953, Reeve and Brown, 1968, Saxton and Jewell, 1969; Szczesniak and Smith, 1969). Additional references can be found in a comprehensive review by Reeve (1970). Chemical and ultrastructural changes result throughout development, harvest, storage and processing of fruits and vegetables. Some of the changes, such as those occurring during ripening or cooking, are desirable from the standpoint of the consumer, textural changes resulting from storage, handling and processing of vegetables are generally undesirable. The textural properties most important to this study are those of crispness and crunchiness.

Vickers (1979) indicated that crispness in wet foods such as celery is a result of the combined properties of rigidity, hardness and firmness. She noted that crunchiness is highly related to crispness, toughness and the bone-conducted sounds occurring during chewing. Vickers related the properties of crispness and crunchiness to structural changes resulting from the rupturing of turgid cells during chewing. The pressure exerted upon the cell wall by the cellular contents, turgor pressure, is dependent upon sufficient water and solutes in cells with rigid cell walls and sound membranes.

Turgor is related to water potential, the free energy of water per mole, in the following equation (Kramer, 1969):

$$\Psi_w = \Psi_s + \Psi_p + \Psi_m \tag{1}$$

where Ψ_w = water potential,
Ψ_s = osmotic potential,
Ψ_p = turgor pressure, and
Ψ_m = matric potential.

Osmotic potential becomes more negative as solute concentration increases. The tendency the matrix has to adsorb water is the matric potential.

Water potential can be related to relative humidity and thus water activity by a formula derived from Raoult's law (Salisbury and Ross, 1978).

$$-\Psi_w = 10.7\ T \log(100/RH) \qquad \text{and} \qquad RH/100 = a_w \tag{2}$$

where T = absolute temperature (Kelvin),
RH = relative humidity, and
a_w = water activity.

There is general agreement with regard to the importance of turgor in fresh texture. Processing of fresh tissue results in many changes which may affect texture. In order to evaluate mechanisms of textural changes during processing, it is necessary to understand the contribution of various cell structures to texture. The relative importance of the cell walls, middle lamellae and cell membranes in texture depends on the tissue. Elasticity, strength, rigidity, etc. of plant tissues are generally attributed to the cell wall (Mohsenin, 1970). However, modulus of elasticity (stress/strain) was correlated to turgor in potatoes (Nilsson et al., 1958). Softness in apples is thought to result from changes in the middle lamella which allow the cells to separate during mastication (Reeve, 1953). The crisp water chestnut (corm of Eleocharis dulcis) maintains crispness in spite of heat processing. Since heat processing destroys membrane integrity (Mirza and Jewell, 1976), crispness in water chestnuts must be a function of firm cell walls or some other structure.

Other cell structures may affect crispness and crunchiness. The tonoplast membrane plays an important role in texture because of the relationship it has to turgor. Most literature relating to cellular stresses emphasizes the plasma membrane rather than the tonoplast or both. This is possibly because of the difficulty of preserving the membrane in cells with large vacuoles and because of its proximity to the plasmalemma. Those processes which affect the plasmalemma would be expected to have a similar effect on the tonoplast.

Sugars, acids, and other solutes of the cytoplasm and vacuole contribute to Ψ_π and are essential for turgor. The Ψ_m is considered small in comparison to Ψ_π and is often ignored. At maximum turgor, $\Psi_w = 0$ so that Ψ_p which is a positive number at full turgor must be offset by approximately equal and opposite Ψ_π from sugars, salts and acids.

Microtubules and the cytoskeleton are in close proximity to the plasma membrane. The relationship between microtubules and the plasma membrane has been reviewed by Weatherbee (1981). Microtubules have been largely ignored in the food texture literature and their role in texture is not understood. Microtubules are not likely to be viewed in many fruits or vegetables because they depolymerize at refrigeration temperatures (Inoue, 1981). Even under the best of circumstances, microtubules are difficult to preserve and view. Since refrigerated fruits and vegetables can maintain their crispness beyond the depolymerization of microtubules, microtubules must not be essential for membrane integrity.

The Effect of Hydration on the Membrane, Cell Wall and Middle Lamella

Changes occurring during dehydration and/or rehydration may alter the structural or compositional properties of cellular membranes. Irreversible loss of membrane integrity may result from damage too extensive to repair, damage to a membrane repair system (Stewart and Bewley, 1982) or interruption of energy transfer (Simon, 1974).

In a comprehensive review on plant membrane permeability, Simon (1974) outlined evidence for mechanisms of membrane leakiness under a variety of conditions. Simon notes that plant membranes become leaky as a result of conditions of chilling, dehydration and senescence. Unlike most plant membranes of biological systems, the membranes of pollen, fungal spores and seeds recover upon rehydration and regain functionality. Pollen, fungal spores and seeds leach at a high rate during the initial stages of rehydration but quickly exhibit a reduced rate of leaching. Simon reviewed evidence that the initial surge of leachate corresponds to a membrane phase change imposed at moisture levels below 20%.

Lipid extracts from human brains at various levels of hydration were evaluated by x-ray diffraction (Luzzati and Hansen, 1962). These authors provide evidence for phase changes from a lamellar phase to a hexagonal II phase (HII) as moisture level was reduced below 20%. The HII appears as inverted micelles with the hydrophilic heads orienting together to form a channel. The hydrophobic tails surround them in a continuous phase. The HII phase has been investigated using ^{31}P nuclear magnetic resonance (Cullis and De Kruijff, 1978) and visualized by freeze-etch electron microscopy (Verkleij et al., 1980, Deamer et al., 1970) and freeze-fracture electron microscopy (Veil and Stollery, 1979). Similarly, Gordon-Kamm and Steponkus (1984) prepared freeze-fractured specimens from protoplasts of nonacclimated *Secale cereale* (rye) leaves after osmotic dehydration, slow freezing and supercooling to the same degree. A lateral phase separation in the plasma membrane, aparticulate lamellae, and HII were observed in osmotic dehydration and slow freezing. Only minor lateral phase separations occurred in supercooling to the same temperature. Because ice formation never took place in supercooled protoplasts, they concluded that the phase change was a result of dehydration and not temperature.

Additional evidence for a phase transition at low levels of hydration has been found in anhydrobiotic organisms. Anhydrobiotic organisms have the ability to survive very low levels of hydration. Seeds, spores, pollen and lichens (Simon, 1974) as well as the anhydrobiotic nematode, *Aphelenchus avenae* (Crowe and Crowe, 1982), have been shown to exhibit increased viability and reduced leaching if, prior to rehydration in a hypotonic solution, they are equilibrated at a higher relative humidity. By Simon's theory, this would allow for a phase change in the membrane from HII to the more restrictive lamellar phase. McKersie and Stinson (1980), however, found the lamellar phase and failed to find the HII in Lotus corniculatus seeds at very low moisture levels.

Comparisons of systems with and without the ability to survive desiccation are useful in understanding dehydration damage. Crowe and Crowe (1982) found that when the anhydrobiotic nematode A. avenae was dried slowly to moisture levels of about 2%, survival was much greater than those which were quick-dried. Biochemical comparisons of the systems revealed that greater quantities of both trehalose and glycerol were produced during slow-drying than in quick-drying, and that the concentration of these solutes correlated well with survival. Another characteristic of this anhydrobiotic system was demonstrated by freeze-fracture. In sarcoplasmic reticulum from quick-dried inviable-nematodes, the phospholipids appeared to be in HII while those of slow-dried viable-nematodes were in laminar form. The authors suggested that trehalose and glycerol prevented the phase change of phospholipids from laminar to HII and improved ultrastructural reconstitution.

Clegg et al. (1982), using the brine shrimp Artemia, presented evidence that supported a water replacement theory for anhydrobiosis. Polyhydroxy compounds replace water at intracellular surfaces in desiccation-resistant organisms. They showed a break in curves of spin-lattice relaxation time, spin-spin relaxation times and self-diffusion for protons when plotted against moisture content. The break occurred in each curve at moisture contents in the range of 20%, suggesting that there was a membrane phase change. The authors hypothesized, that at low water contents when only a few layers of water molecules remain next to surfaces, polyhydroxy compounds replace water at these surfaces and the "bound" water becomes more "free."

Although evidence supports the existence of a membrane phase change at water contents below 20% moisture, this mechanism of damage cannot be used to explain irreversible wilting, which occurs at relatively high moisture contents in desiccation-intolerant plants. Graziani and Livne (1971) dehydrated tobacco leaf disks to various levels and then floated them on water. Using rate of rehydration and leaching as indicators, they found irreversible membrane damage when the tissue was reduced to 40% of fresh weight or less. Mir et al. (1969) equilibrated corn roots at various relative humidities and found that, when 75% of the water had been lost, they were no longer viable.

Shipman et al. (1972) suggested that damage to dehydrated tissue may also occur during rehydration. Stasonkus et al. (1981) reviewed literature with regard to membrane damage during thawing, which is analogous to rehydration. They cited observations that, after hypertonic contraction in a cell has occurred, the exposure of the cell to a hypotonic environment causes it to rapidly expand and lyse. Similarly, Morris and McGrath (1981) photographed a freeze-thaw cycle in liposomes. Upon thawing there was an increase of vesiculation. They also made liposomes with trapped glucose and placed them in various hypertonic solutions of NaCl. As the molarity of the salt solutions increased, so did the release of glucose. There was a much larger flux of glucose from the liposomes following resuspension into the isotonic solution than during initial contraction. Following lysis there was a resealing of the liposome and restitution of osmotic responsiveness.

A hypothesis which is often represented in the literature is that of minimum volume as proposed by Meryman (1968, 1971). Meryman plotted hematocrit readings against concentration of NaCl and encountered an apparent minimum volume at about 0.8 M NaCl. He concluded that at that volume the plasma membrane incurred damage which rendered it leaky to surrounding solute thereby preventing further volume reduction. Wiest and Steponkus (1979) replotted the data of Meryman using a van't Hoff plot (volume versus 1/osmolality) and produced straight lines, indicating that there was no minimum volume over the concentrations used. They believed damage might be more closely related to changes in the membrane surface area than volume of the cell. In an ingenious experiment, Wolfe and Steponkus (1981) separated the effect of volume changes and changes in membrane surface area. They connected a micropipette to a manometer and towed an individual protoplast through an osmotic gradient. They were able to control the surface area by sucking a portion of the protoplast into the pipet. They reduced the volume and kept the surface area constant in one trial and in another they reduced both the volume and the surface area simultaneously. They found that the viability of the protoplasts correlated to a specific surface area of the membrane, regardless of the volume.

Steponkus and co-workers (Dowgert and Steponkus, 1984, Steponkus et al., 1983, Wiest and Steponkus, 1979) reported the changes in surface area over various osmotic regimes. Protoplasts of spinach and rye were hypotonically expanded to the point of lysing. The membranes were surprisingly elastic and increased 1.24 times before 50% of the protoplasts lysed. Protoplasts first hypertonically contracted (HCP), however, to a fractional surface area of 0.56, were only able to recover a fractional surface area of 0.78 before expansion-induced lysis occurred. From this work they developed the term "tolerable surface area increment" (TSAI) and TSAI50, the latter of which denotes the surface area increment tolerated by 50% of the cells. They noted that acclimated cells had a greater TSAI50 than did nonacclimated cells and concluded that this increased resilience was at least one adaptation explaining survival. Steponkus et al. (1983) presented electron micrographs comparing acclimated and nonacclimated cells. Nonacclimated HCP had a greater number of endocytotic vesicles than did acclimated cells. On the other hand, hundreds more exocytotic vesicles were observed on the surface of acclimated HCP than on nonacclimated HCP.

Jensen and Oettmeier (1984) presented evidence indicating that dehydration damage is a result of the increased concentration of solutes to the point of toxicity. They proposed from their study of chloroplast membranes that channels are created in the membrane by the local action of concentrated salts and/or cryotoxicants. They provided evidence that the ATP synthetase enzyme was denatured at high solute concentrations.

Changes in permeability may result from lipid oxidation or from formation of free fatty acids. Bouzas and Karel (1984) used red blood cells for a model and proposed that oxidation of the membrane under dry storage conditions may be responsible for protein and phospholipid changes. Polymerization of membrane proteins and oxidation of membrane lipids would be expected to affect permeability. However, there was almost no evidence of these reactions at the onset of storage. Because irreversible changes in texture occur during dehydration of plant tissue, well before storage, other mechanisms are most likely responsible for changes occurring during dehydration.

Senaratna et al. (1984) evaluated x-ray diffraction patterns of microsomal membranes from soybean axes at 6 hr of imbibition (desiccation-tolerant stage) and 36 hr of imbibition (desiccation-intolerant stage). They measured an increase in free fatty acids, which they correlated to an increase in the phase transition temperature of the microsomal membranes from 7C to 47C. Mabrey and Sturtevant (1977) measured a similar increase of about 20C in the transition temperatures of purified phospholipids in multilamellar aqueous suspensions when free fatty acids of equal chain length were added to the system. Membrane phase change to a gel would certainly explain loss of differential permeability. This mechanism, however, cannot be used to explain situations where there are no measurable changes in the composition of the membrane.

Stewart and Bewley (1982) compared lipoxygenase activity and changes in phospholipid saturation between desiccation-tolerant *Tortula ruralis* and desiccation-intolerant moss *Cratoneuron* filiclnum. Tortula ruralis exhibited lower lipoxygenase activity during dehydration and remained low in rehydration, whereas lipoxygenase activity in desiccation-intolerant moss C. filicinum increased to the level of the undesiccated control shortly after rehydration. They postulated that suppressed lipoxygenase activity in T. ruralis aided in its ability to revive upon rehydration. They also measured a greater increase of unsaturated fatty acids during rehydration of C. filicinum than T. ruralis. They could not, however, show any correlation between phospholipid saturation and membrane permeability.

Sterling (1963) and Reeve (1970) attributed a great deal of importance to the middle lamella and texture. Despite the importance of this intercellular cement, not much attention has been given to its fate during dehydration. Because it is composed of highly hydrated pectic polymers, dehydration would be expected to adversely affect its stability.

Holdsworth (1971) attributed the textural changes resulting from dehydration to an increase in cell wall crystallinity. Crystallinity is the extent to which the molecule exhibits well-defined and symmetrical planes. The effect that dehydration has on this highly hydrated matrix of cellulose, hemicellulose, pectins, etc. has been studied (Sterling and Shimazu, 1961; Shimazu and Sterling, 1961). Using x-ray diffraction, enzymatic degradation, iodine tests and other methods, they showed that cellulose crystallinity increases upon dehydration and that the increase is irreversible. They describe the increase in crystallinity as an increase in polysaccharide-polysaccharide bonds replacing polysaccharide-water bonds, as water is removed. As the crystallinity of the cell wall increases, the ability it has to rehydrate decreases. The authors attributed loss of fresh texture in dehydration to the failure of the cell wall to take up water and regain its original shape.

Quality Retention and Dehydration of Celery

Anhydrobiosis, the ability to survive desiccation, is a property of some plants (Hallam and Gaff, 1978), some animals (Clegg et al., 1980, Crowe and Crowe, 1982), and seeds (Simon, 1974). Celery does not exhibit that ability and is irreversibly changed during water removal. Attempts have been made both to understand this damage and to minimize it.

Freeze drying (Neuman, 1972), polyacrylamide crosslinking (Schwimmer, 1969), and puff drying (Wilson, 1965) have been employed as other means of avoiding collapse of cell walls. Upon rehydration, celery crescents regained much of their fresh weight and volume. However, these drying techniques resulted in textural changes which render them soft and flaccid (Neuman, 1972).

Osmotic dehydration and infusion of tissue with low molecular weight osmotica have been used with some success (Ponting et al., 1966). Rahman et al. (1976) produced an intermediate moisture celery by treating celery pieces with various concentrations of glycerol prior to air drying at 43C. The pieces infused with glycerol were more rigid and more acceptable than freeze dried, puff dried or untreated hot-air dried pieces, but were only about 33% as rigid and were less acceptable than fresh celery. Solute contamination and loss of soluble solids are also problems associated with infusion and osmotic dehydration.

Camirand et al. (1968) used a calcium pectate membrane to protect several food systems from solute contamination and loss of soluble solids during osmotic dehydration in sucrose. The combination of the protective membrane and osmotic dehydration resulted in tissues with physical and organoleptic properties that somewhat resembled those of fresh tissue. They reported that the membrane was more effective in both avoiding solute contamination and loss of soluble solids for animal systems than for plant systems.

Water removal affects many aspects of cell structure and thereby changes texture. The purpose of this study was to identify and evaluate changes at the structural and compositional level in order to understand the mechanism of irreversible textural changes during dehydration, as postulated in the following section on theory formation.

THEORY FORMATION

Cell Wall and Middle Lamella Crystallinity

Propositions

1. Crystallinity of cell wall and middle lamella polymers increases during dehydration (Sterling and Shimazu, 1961).

2. Ethanol solutions destroy the differential permeability of cell membranes (Grisham and Barnett, 1973, Hubbel et al., 1970).

3. Modulus of elasticity can be measured by the Instron Universal Testing Machine as an indication of rigidity (Mohsenin, 1970).

Assumptions

1. The process of moisture removal by exposure to various concentrations of polyethylene glycol of known a_w's produces uniformly dehydrated tissue without the complicating factors of temperature in hot-air dehydration. The loss of solutes to the surrounding solution may have an effect on texture.

2. Ethanol treatments can be used to separate the role of the surrounding intact cell walls and middle lamella from the role of the membrane in celery texture.

3. Changes in cell wall and middle lamella crystallinity can be observed using transmission electron microscopy.

4. Dehydration of celery tissue using the conventional hot-air method can be used to produce a standard of crystallinity in the cell wall and middle lamella. Crystallinity may appear as clumping of microfibrils in the cell wall and as loss of cementing ability in the middle lamella.

Inference

The role of the cell wall and middle lamella crystallinity on texture can be investigated by relating the crystallinity detected in electron microscopy with the modulus of elasticity from the Instron Universal Testing Machine at various water activities. Information from tissue with membranes altered in 20% ethanol should be useful in separating, at least in part, the role of the cell wall and middle lamella from that of the tonoplast and plasma membranes.

Biophysical Changes in the Plasma and Tonoplast Membranes

Propositions

1. Membrane permeability is altered by dehydration (McKersie and Stinson, 1980) and leakage of cell solutes is an indication of permeability (MacRobbie, 1971).

2. Ethanol solutions destroy the differential permeability of cell membranes (Grisham and Barnett, 1973; Hubbel et al., 1970).

3. Modulus of elasticity as measured by the Instron Universal Testing Machine is an indication of rigidity (Mohsenin, 1970).

4. Fine structure of plant tissue at various levels of dehydration may be examined with transmission electron microscopy (Hallam and Goff, 1978).

5. Maximum cell volume occurs at full turgor. Differences in turgor are reflected in cell size and consequently volume of the tissue (Vickers, 1979).

Assumptions

Damage to the cell membrane during moisture removal can be examined with regard to

a. leakage from tissue prepared at various water activities, and
b. the point in moisture removal where damage to the plasma membrane and/or tonoplast is irreversible.

The preceding observations will be compared with textural measurements to determine correlation between texture and the condition of the plasma membrane and tonoplast.

Inference

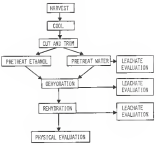
By evaluating evidence of membrane damage, using turgor, modulus of elasticity, leakage and electron microscopy, the level of moisture removal where damage is irreversible can be determined.

MATERIALS AND METHODS

Scope of Work

Figure 4 outlines the experimental design of this study. An overview of the experimental design will follow. Celery petioles were harvested, cooled in the field, and stored in a cooler until sample preparation. At the time of sample preparation, petioles were cut into 0.5 cm cross-sections (crescents). In order to separate the effect of cellular membranes from cell walls on texture, a treatment was devised which impaired membrane integrity but had no apparent effect on wall integrity. Half the celery crescents were pretreated with an ethanol solution, which impaired membrane integrity, and half were pretreated with distilled deionized water (DDH_2O). After the pretreatment, samples of the bathing solutions were taken for leachate evaluation to determine the effectiveness of the plasma and tonoplast membranes as diffusion barriers, and the crescents were osmotically dehydrated in eight concentrations of polyethylene glycol 8000 (PEG) of known water activities (a_w's). A total of 16 treatments, 8 osmotica times 2 pretreatments, were used. Fresh celery crescents were hot-air dried for comparison. Minerals, amino acids and conductivity were measured in the dehydration solution as an indication of membrane integrity. The crescents were then rehydrated in DDH_2O, after which the rehydration solution was also evaluated for

Figure 4. Flow diagram of experimental procedure.


HARVEST
COOL
CUT AND TRIM
PRETREAT ETHANOL
PRETREAT WATER
LEACHATE EVALUATION
DEHYDRATION
LEACHATE EVALUATION
REHYDRATION
LEACHATE EVALUATION
PHYSICAL EVALUATION

minerals, amino acids and conductivity. Modulus of elasticity and piece height of the rehydrated crescents was measured for each of the 16 treatments. Comparisons between modulus of elasticity, ultrastructure and membrane integrity were made between all 16 of the treatments and a hot-air dried treatment.

Sample Preparation and Treatments

At each preparatory step attempts were made to minimize sample variation. Similar sized celery petioles (1.5-2.5 cm diameter) of the variety (M-68-29) grown in the same area were harvested from a major Florida grower in Zellwood (A. Duda and Sons), trimmed and placed on ice in coolers in the field. The harvest took place in the morning to minimize field heat. Petioles were transported to the University of Florida, Gainesville, and stored leaf end up in loosely closed containers at 1.7C until experimental use. The following day, celery petioles of similar thickness (1.5-2.5 cm diameter) and color were selected from 3 to 4 plants, cleaned and trimmed. Crescents of 0.5 cm thickness were made from the middle portion using a cutter with sharp replaceable blades affixed 0.5 cm from a face plate. Two additional runs using the same lot of celery followed at 2 week intervals. A run consisted of preparation, pretreatment, dehydration, rehydration and the analyses of crescents and leachates.

Pretreatment

In order to determine the effect of membrane integrity (integrity of tonoplast and plasma membrane) a pretreatment of 20% ethanol, which has been shown to impair membrane integrity (Grisham and Barnett, 1973), preceded dehydration. Grisham and Barnett (1973) used electron spin resonance to compare the effect of various alcohols on the membranes of lamb kidney cells. For every alcohol tested the concentration of alcohol required to inhibit $(Na^+ + K^+)$-ATPase correlated with the inability of the membrane to orient an androstanol probe. Maximal perturbations were induced by ethanol concentrations of 20%. Using the androstanol probe, they concluded that denaturation of the ATPase resulted from changes in the lipid structure rather than from a direct interaction of the alcohol and the membrane protein. If ethanol were effective in disrupting membrane structure (as it was shown to be in the case of sheep kidney cells) of celery petiole cells without altering cell walls and the middle lamellae, then the effect of membrane integrity on physical properties could be measured.

Sixteen groups of 20.5 ± 0.5 g each (about 20 crescents) of fresh crescents were accurately weighed and placed in each of 16 Fleakers (Corning, Corning, NY) half of which contained 250 g of 20% ethanol and half of which contained 250 g DDH_2O. A shaker continuously agitated the Fleakers in a circular motion at about one revolution per sec for 5 hr (estimated penetration time) at 5C. Penetration time of ethanol was estimated by using 2 drops of an ethanol soluble stain, 1% methylene blue, in the bathing solution of a test flask containing 100 ml of 20% by weight ethanol and 5 crescents. The crescents were entirely blue after 5 hr.

The crescents were removed from the pretreatment Fleaker with a nylon net, blotted on ashless filter paper and weighed immediately in preparation for the dehydration step. Crescents in one of the DDH_2O pretreatments and in one of the ethanol pretreatments were maintained in DDH_2O throughout dehydration and rehydration. Crescents and leachates from these Flenkers were used to evaluate the effect of pretreatment.

Evaluation of Pretreatment Effect on Membrane Integrity

After pretreatment, leachate aliquots of 40 g were taken from each flask, filtered using 0.45 μ Alpha Metricel 450 filters (Gelman, Ann Arbor, MI), divided between two 20 ml plastic scintillation vials and stored at 5C until use (not more than 5 days).

Leachate evaluation as an indication of membrane integrity consisted of conductivity, K, Na, Mg, Ca and amino acid measurements. Conductivity was measured using a YSI Model 31 conductivity meter (Yellow Springs, OH). Mineral solutions were prepared by accurately weighing approximately 20 g of each of the leachate samples and fresh-tissue samples and oven drying them in 50-ml Pyrex beakers. The beakers were transferred to a muffle furnace and held at 300C for 1 hr followed by 600C for 10 hr or until only white ash remained. Samples with higher concentrations of PEG were more difficult to ash and required more time at 600C. After cooling, the ash was dissolved in 2 ml of concentrated HCl and then brought up to 10 ml with DDH_2O, mixed and transferred to 20 ml acid-cleaned plastic vials in preparation for mineral analysis at the University of Florida Extension Soil Testing Laboratory. Flame emission was used for Na and K and atomic absorption, for Mg and Ca, according to the methods of Mitchell and Rue (1978). Dry weight of celery was measured by accurately weighing approximately 10 g of tissue, drying the tissue for 6 hr at 100C, then for 6 hr at 100C and -172 MPa. The dried tissue was then cooled in a desiccator and analytically weighed. Percent of total content of each component in the leachate was calculated by dividing the observed µg components/g dry celery in the leachate over the total µg's component/g dry celery in fresh celery.

Free amino acid concentration was determined using an orthopthalaldehyde (OPA) method (Anon., 1983b) modified for spectrophotometry. Since OPA complexes with primary amines to produce a fluorophor (Benson and Hare, 1975), it also reacts with peptides and proteins. The measurements made were considered to be relative estimates of amino acid flux and not absolute measurements. A spectrophotometric scan was used to confirm 340 nm as the absorbance maximum of the OPA-amino acid complex (Roth, 1971). Distilled deionized water was used as a blank, and standards of L-leucine were made ranging from 0.025 µM/ml-0.75 µM/ml. Concentration was calculated from the equation of the line fit to the standards (r^2 = 0.996). Total amino acids in celery were determined by homogenizing equal weights of boric acid buffer and tissue in a high speed Waring blender for 3 min after which the homogenate was centrifuged at 10,000xg for 30 min. The supernatant was then filtered in the same manner as the leachates were. Amino acid content was reported as percent leached of total amino acids or 100 x µM leached/g dry celery over total µM/g dry celery.

Pretreatment means were calculated for the percent leached of total for amino acids, conductivity and minerals. The means were compared using t-intervals ($\alpha/2 = 0.025$) (Ryan et al., 1982).

Ultrastructural observations were made on celery tissue from both pretreatments and fresh tissue fixed in the field using the methods described in the dehydration and rehydration section. Comparisons of membrane integrity and cell wall structure were made between pretreated tissue and fresh tissue.

Evaluation of Pretreatment on Physical Properties

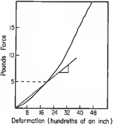
Modulus of elasticity taken at the initial straight line portion of the stress strain curve (Mohsenin, 1970) is indicative of stiffness and rigidity. When the stress strain curve is not linear, the slope of the line drawn from the origin to a predetermined point on the stress strain curve (secant modulus) may be evaluated (Mohsenin, 1970). In this study secant moduli were calculated using the slope of the line drawn from the origin to the intercept at 5 lb (22.2 N) on the force versus deformation curve (Figure 5) in the following equation:

$$\frac{\text{Stress}}{\text{Strain}} = \frac{F/A}{d/L} = \text{Modulus of elasticity} \qquad (3)$$

where F = force applied to the crescent by the anvil,
A = cross-sectional area of the crescent in contact with the anvil,
d = crescent deformation (distance traveled by the anvil),
L = initial crescent height.

Force versus deformation curves were made using an Instron Universal Testing Machine (Canton, MA). The Instron was calibrated at

Figure 5. Typical force deformation curve for intermediate moisture celery.


Pounds Force
15
10
5
8
16
24
32
40
48
Deformation (hundreths of an inch)

full scale reading of 444.8 N (100 lb) with a chart speed of 5.08×10^{-3} M/sec and a crosshead speed of 8.47×10^{-5} M/sec (0.2 in/min) using an anvil of 5.7×10^{-2} M in diameter.

Because cell size decreases when turgor decreases, crescent height was expected to be affected by any change in turgor. Calipers were used to measure piece height following pretreatment. Means (from 17-20 crescents/pretreatment) of modulus of elasticity and piece height were made for each of the pretreatments. Pretreatment means were compared using t-intervals ($\alpha/2 = 0.025$) (Ryan et al., 1984).

Dehydration and Rehydration

Osmotic dehydration was selected as a method of water removal because temperature effects could be avoided and final a_w could be controlled. Osmotic dehydration is typically done with solutions of small molecules such a sucrose. Small molecules, however, contaminate the tissue. Polyethylene glycol 8000 (PEG) (Union Carbide, Newark, NJ) is a highly soluble polymer and can be mixed with water to produce solutions of known a_w (McClendon, 1971). Using the criteria of Carpita et al. (1979), PEG 8000 was shown to draw moisture from the cell without penetrating the cell wall. Eight osmotica were made according to Table 1, as determined by McClendon (1971) and converted by equation 2. McClendon's values were very close to those determined in this study using Wescor Soil Psychrometers (Logan, UT).

Both ethanol and DDH$_2$O pretreated samples (a total of 16 Fleakers) were transferred to 500 g of the eight osmotica in 1000 ml

Table 1. Water activity of various solutions of PEG.

	Concentration of PEG (g/1000 g H_2O)							
	0	100	250	400	500	600	800	1000
a_w	1.000	0.999	0.995	0.987	0.980	0.970	0.948	0.920

Fleakers and dehydrated for 13 hr at 22C as described in the pretreatment. In preliminary studies, crescents dehydrated approximately 13 hr in 0.920 a_w before no weight change was detectable. The equilibrated samples were retrieved from the PEG solution with a nylon net, rinsed 10 sec in DDH_2O to free them from the viscous PEG, blotted on ashless filter paper and weighed. The rinsing and blotting removed the majority of the PEG. Percent weight retention upon dehydration (100 x g dehydrated tissue/original g fresh tissue) was calculated and t-intervals for the values were made ($\alpha/2 = 0.025$) (Ryan et al., 1982). Leachate samples were taken and treated as described in the pretreatment section.

The partially dehydrated samples were then rehydrated in 250 g DDH_2O at 5C in 8 hr (the time required to bring crescents to a constant weight), using continuous agitation, as in the pretreatment. Crescents were blotted, weighed and 40 g leachate samples were taken and treated as described in the pretreatment section. Percent weight recovery upon rehydration (100 x g rehydrated tissue/original g fresh tissue) was calculated and t-intervals for the values of the 3 runs were made ($\alpha/2 = 0.025$).

Evaluation of Dehydration and Rehydration Effect on Membrane Integrity

Leakage and membrane appearance as visualized using transmission electron microscopy (TEM) were used as parameters of membrane integrity. Mineral composition in leachate and fresh celery was evaluated as described in the pretreatment section. Conductivity and amino acid concentration were also measured as described above. Separate conductivity standard curves were made for each PEG solution by using various concentrations of NaCl. The values were used to correct for the conductivity of PEG solutions. Each of the components was reported as a percentage of the maximum observed leakage during dehydration.

The mean response of each leachate component versus concentration of PEG (a_w) were compared for dehydration leachates from ethanol pretreated samples and DDH$_2$O pretreated samples using linear regressions (Freund and Littell, 1981). The differences between the mean responses of the pretreatment lines were tested to determine if they were significant, and the slopes were tested to determine if they were significantly different from 0.0.

Plots of concentration of each leachate component in the rehydration solution versus the a_w at which the crescents were previously dehydrated were made. Leachate concentration for each treatment was compared to the control using a one-sided t-test ($\alpha = 0.05$) (Ryan et al., 1982).

Crescents from each of the 16 treatments were fixed 2.5 hr in 0.5 strength Karnovsky's (1965) fixative followed by 2.5 hr in 2% OsO$_4$ in cacodylate buffer (0.1 M-pH 7.2) (Hayat, 1981). The tissue was dehydrated in an ethanol series (15 min in 25%, 15 min in 50%, 45 min in 75% with 2% uranyl acetate, 3 rinses in 75%, 15 min in 95%, 15 min in 100%, 45 min in 100%), then in an acetone-Spurr's resin series (Spurr, 1969) prior to hardening at 60C for 9 hr. Sections of 70-90 nm (silver to gold) were made using glass knives on an LKB ultratome 111. Ultrathin sections received 2% uranyl acetate then lead citrate poststains (Reynolds, 1963), each for 0.5 hr. Observations and micrographs were made using a Hitachi HU 11E or Phillips 301 microscope operated at 75 and 60 KV, respectively. Micrographs selected were representative of observations of approximately 80 cells.

Evaluation of Dehydration and Rehydration Effect on Physical Properties

As soon as rehydration was complete, each celery crescent was carefully placed on an ink blotter and an impression was made on a white sheet of paper. The impression was later traced with a polar planimeter to determine cross-sectional surface area. The final height was measured using calipers.

Crescents were hot-air dried to about 6% moisture in order to obtain prepared walls of increased crystallinity (Sterling and Shimazu, 1961) for physical and ultrastructural comparisons.

Modulus of elasticity of the crescents was measured using the Instron Universal Testing Machine. Instron data analysis was identical to that described for pretreated crescents. The means and t-intervals ($\alpha/2 = 0.025$) of modulus of elasticity were calculated from 17-20 crescents from each treatment (Ryan et al., 1984).

Tissue from hot-air dried crescents was prepared for TEM as was tissue from crescents of each pretreatment. The same preparation for TEM was used for cell wall observation as was used for membrane integrity with the exception of the addition of 0.15% ruthenium red to both fixes. Alternatively, the ultrathin sections were poststained according to the method of Hoch (1977), which enhances visualization of polysaccharides of the cell wall. Micrographs selected for presentation are representative of at least 80 observed cells. Cell walls and middle lamellae were examined in tissue from each treatment on a Hitachi HU 11E or a Phillips 301 electron microscope for signs of crystallinity, such as irregular wall thickness and clumping of the microfibrils.

RESULTS AND DISCUSSION

Ultrastructural and physical changes which occur as a result of dehydration and rehydration of celery were evaluated. The contributions of the cell membrane and cell wall to rigidity were separated and a level of a_w was determined below which water could not be removed and then added back to obtain rigidity similar to that in fresh tissue.

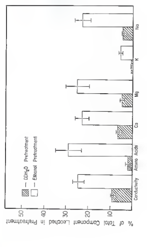
Effect of Pretreatments

Crescents pretreated in ethanol were visually similar to those pretreated in DDH_2O. Crescents pretreated in ethanol were, however, more pliable, appeared a slightly deeper green and reflected less light than crescents pretreated in DDH_2O.

Effect of Pretreatment on Membrane Integrity

Leachates from crescents pretreated with 20% ethanol were compared to leachates from crescents pretreated with DDH_2O (Figure 6). Two and a half to 6 times more of each leachate component was measured in the bathing solutions when crescents were pretreated with 20% ethanol. Crescents pretreated in DDH_2O leached very low quantities into the bathing solution. This indicated that ethanol was effective in disrupting cell membrane function. Losses of the various components ranged from 5-40% of the total component in the tissue.

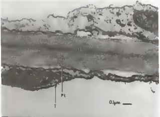
Figure 6. Comparison of leachate components leached into the pretreatment bathing solutions. Bars indicate t-intervals from 8 replications.

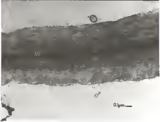

▨ — DDH₂O Pretreatment
☐ — Ethanol Pretreatment
% of Total Component Leached in Pretreatment
50
40
30
20
10
Conductivity
Amino Acids
Ca
Mg
K
Na

A larger portion of the minerals might be expected to be lost to the bathing solution, if there were no barriers to diffusion. However, if the ethanol pretreatment resulted in an increased formation of vesicles, some of the minerals and amino acids, etc. could be trapped in these vesicles and prevented from leaching into the bathing solution. Alternatively ethanol may render the membrane "leaky," but not totally ineffective as a diffusion barrier. In this case, equilibration between cellular contents and the bathing solution might take substantially longer than the 5 hr pretreatment period.

Cell wall junctions of DDH_2O and ethanol-pretreated tissues are compared in Figure 7. Tissue from fresh crescents had intact cell walls and middle lamellae with no evidence of wall collapse and almost no distortion. Most of the cells observed had intact plasma, tonoplast and organelle membranes. Likewise, most cells in tissue that was DDH_2O pretreated and not dehydrated exhibited these characteristics. However, good preservation of more cells resulted when tissue was fixed in the field than when tissue was fixed after DDH_2O treatments. The latter tissue received a treatment at 1.000 a_w and plasmolysis in the fixative may be an explanation for the difference in the numbers of cells with well-preserved plasma and tonoplast membranes. Integrity of the cell wall microfibrils and middle lamellae appeared unchanged by the DDH_2O or ethanol pretreatment. Since ethanol may precipitate pectins, it may have had an effect on the cell wall which was not detected. Distortion of membranes and cytoplasm into vesicles was common in ethanol-pretreated tissue and, in many sections of cells, all that remained was an empty

Figure 7. Comparison of cell wall junctions of DDH_2O-pretreated tissue (above) and ethanol-pretreated tissue (below). Microfibrils appear in transverse cross-section (above) and longitudinal section (below). C = cytoplasm, ML = middle lamella, PL = plasmalemma, CW = cell wall, and T = tonoplast.


CE
MT
CW
PL
T
0.1μm


CW
MT
0.1μm

cell wall. In no case did tissue pretreated with ethanol exhibit intact plasma membranes, tonoplasts or cytoplasm. The extensive disruption of membrane systems observed in ethanol-pretreated tissue may be a direct result of the pretreatment or may have resulted during fixation, after the membranes were predisposed to further damage by the ethanol pretreatment. The results of TEM and leachate evaluation indicate that 20% ethanol impaired membrane function, but are not conclusive evidence as to the nature of the damage.

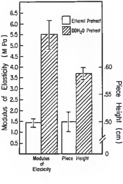
Effect of Pretreatment on Physical Properties

Loss of membrane integrity also resulted in physical changes. Modulus of elasticity, as measured by the Instron Universal Testing Machine, was 5.5 ± 0.7 MPa for DDH$_2$O-pretreated crescents and less than a third of that value (1.4 ± 0.2 MPa) for ethanol-pretreated crescents. Piece height was significantly lower when samples were pretreated with ethanol (0.55 ± 0.07 cm) than with DDH$_2$O (0.59 ± 0.01 cm) (Figure 8). Since in TEM examination of DDH$_2$O-pretreated and ethanol-pretreated crescents neither the middle lamella nor the cell wall exhibited distortion or clumping (Figure 7), it is likely that changes in membrane integrity were mainly responsible for the observed physical changes.

Dehydration and Rehydration

Crescents from both ethanol and DDH$_2$O pretreatments, dehydrated to an a_w of 0.920, were shrunken. Crescents treated only with DDH$_2$O and not dehydrated had a fresh firm appearance. After rehydration, crescents dehydrated to the lower a_w's recovered about 80% of their original fresh weight and were flaccid, but did not appear shrunken.

Figure 8. Comparison of pretreatment effect on modulus of elasticity and piece height. Bars indicate t-intervals from 17-20 observations.


Modulus of Elasticity (M Pa)
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Ethanol Pretreat
DDH$_2$O Pretreat
.60
.55
.50
0
Piece Height (cm)
Modulus of Elasticity
Piece Height

Effects of Controlled Water Removal on Membrane Integrity

The effect of dehydration on leakage for each pretreatment is summarized in Figures 9-14. If dehydration impaired membrane integrity as ethanol pretreatment was shown to do, an increase in leachate components would be expected as dehydration progressed to lower levels of a_w or at a critical a_w where membrane damage might occur. For each of the pretreatments, amino acids and conductivity did increase slightly as a_w decreased (Figures 9 and 10). However, there was no "critical a_w" below which leachate concentration increased and at no point did the concentration of amino acids and conductive components in the leachate of DDH$_2$O-pretreated crescents reach the levels observed in the leachate of ethanol-pretreated crescents. The slight increase in leachate concentration in the bathing solution of DDH$_2$O-pretreated crescents may represent losses resulting from the osmotic gradient and may not reflect changes in membrane permeability.

Because of large standard errors of the means, pretreatment effect on mineral loss during dehydration appeared to be small (Figures 11-14). Larger than expected errors were likely the result of mineral contaminants in the PEG, which were close to the range of the leachates being tested. In addition, samples containing PEG left residues in the ashing container, which were insoluble in water or concentrated HCl and may have trapped minerals within them. If ethanol pretreatment caused vesicle formation, it is also possible that the extent of vesicles formation from cell to cell would vary greatly and produce considerable variation in leachate concentration.

Figure 9. Effect of a_w on leaching of amino acids from crescents pretreated in DDH_2O or ethanol. Bars indicate standard error of the mean for 3 replicates.

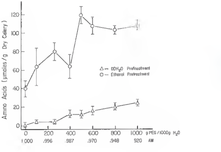

Amino Acids (μmoles/g Dry Celery)
120
100
80
60
40
20
0
△— DDH$_2$O Pretreatment
○— Ethanol Pretreatment
0
200
400
600
800
1000
g PEG/1000g H$_2$O
1.000
.996
.987
.970
.948
.920
AW

Figure 10. Effect of a_w on leaching of conductive components from crescents pretreated in DDH_2O or ethanol. Bars indicate standard error of the mean for 3 replicates.

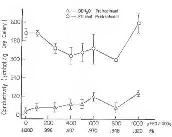

△ — DDH$_2$O Pretreatment
○ — Ethanol Pretreatment
Conductivity (µmhol / g Dry Celery)
600
480
360
240
120
0
0
200
400
600
800
1000
gPEG/1000g H$_2$O
1.000
.996
.987
.970
.948
.920
AW

Figure 11. Effect of a_w on leaching of calcium from crescents pretreated in DDH_2O or ethanol. Bars indicate standard error of the mean for 3 replicates.

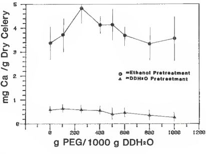

mg Ca /g Dry Celery
0
1
2
3
4
5
0
200
400
600
800
1000
1200
g PEG/1000 g DDH₂O
○ =Ethanol Pretreatment
▲ =DDH₂O Pretreatment

Figure 12. Effect of a_w on leaching of sodium from crescents pretreated in DDH_2O or ethanol. Bars indicate standard error of the mean for 3 replicates.

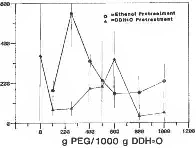

µg Na /g Dry Celery
0
200
400
600
○ =Ethanol Pretreatment
▲ =DDH₂O Pretreatment
0
200
400
600
800
1000
1200
g PEG/1000 g DDH₂O

Figure 13. Effect of a_w on leaching of magnesium from crescents pretreated in DDH_2O or ethanol. Bars indicate standard error of the mean for 3 replicates.

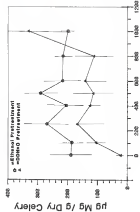
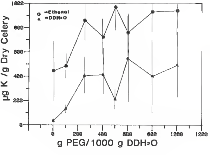

○ =Ethanol Pretreatment
▲ =DDH₂O Pretreatment
µg Mg /g Dry Celery
400
300
200
100
0
0
200
400
600
800
1000
1200
g PEG/1000 g DDH₂O

Figure 14. Effect of a_w on leaching of potassium from crescents pretreated in DDH_2O or ethanol. Bars indicate standard error of the mean for 3 replicates.


µg K /g Dry Celery
1000
800
600
400
200
0
○ =Ethanol
▲ =DDH₂O
0
200
400
600
800
1000
1200
g PEG/1000 g DDH₂O

Some information may be gained by comparison of the mean response of the data in Figures 9-14. The mean response for each of the minerals were significantly higher for leachates from ethanol-pretreated crescents than from those of DDH$_2$O-pretreated crescents (Table 2). The greater response suggests that the diffusion barrier in ethanol-pretreated crescents is less effective than that of DDH$_2$O-pretreated crescents at $a_w = 1.000$. In general the slopes of lines fit to these data were not significantly different from 0.0. The exception to this is the case of amino acids where both the ethanol and DDH$_2$O-pretreated crescents leached greater quantities as a_w decreased. The slope of the ethanol curve was 0.065 and that of the DDH$_2$O was 0.025. This suggests that a decreasing a_w had a greater effect on leaching of amino acids when crescents were pretreated with ethanol than with DDH$_2$O. It is not known why leaching of amino acids followed this pattern, whereas there appeared to be no uniform pattern for leaching of the other components. It is assumed, however, that the small number of replications and the large errors are responsible in part for the differences, and that the leaching of the other components would appear similarly if more replications were done.

Additional information about the status of the plasma membrane can be obtained from a comparison of dehydration weight retentions (percent of initial fresh weight after dehydration) (Table 3). The most severe drying at 0.820 a_w resulted in weight retentions of 22.3% for DDH$_2$O-pretreated crescents and 7.3% when crescents were pretreated with ethanol. The greater retention of amino acids, conductive components, minerals and water (as indicated by weight retention) is

Table 2. Comparison of pretreatment effect on leaching of various components. Mean responses of plots at concentration of various leachate components versus PEG concentration were made. The effect of pretreatment was statistically compared.

Leachate component	Pretreatment	Mean Response	Pr>|t|[a]
Conductivity	DDH_2O	61.52 μmho	<0.001
	Ethanol	383.38 μmho	
Calcium	DDH_2O	4.76 μg/g	<0.001
	Ethanol	38.48 μg/g	
Magnesium	DDH_2O	-[b]	-
	Ethanol	-	
Potassium	DDH_2O	-	-
	Ethanol	-	
Sodium	DDH_2O	-	-
	Ethanol	-	
Amino Acids	DDH_2O	11.84 μmoles/g	<0.001
	Ethanol	85.30 μmoles/g	

[a] Pr>|t| for comparison of mean response of DDH_2O and ethanol pretreatments.

[b] Indicates data with errors too large to make comparisons meaningful.

Table 3. Mean percent fresh weight for celery crescents equilibrated at various a_w's. T-intervals are given.

	Pretreatment			
	DDH_2O		Ethanol	
a_w	Dehydration weight retentions	Weight recovery upon rehydration	Dehydration weight retentions	Weight recovery upon rehydration
1.000	107.48 ± 4.11	109.47 ± 3.95	91.10 ± 1.50	91.98 ± 1.07
0.999	102.58 ± 3.36	107.20 ± 1.85	14.39 ± 2.69	50.77 ± 15.48
0.995	84.38 ± 27.53	107.90 ± 0.99	16.81 ± 7.13	72.68 ± 11.27
0.987	54.82 ± 4.02	81.58 ± 15.49	14.95 ± 3.46	72.60 ± 11.85
0.980	43.07 ± 4.62	84.33 ± 4.96	12.28 ± 1.90	68.01 ± 16.88
0.970	32.26 ± 1.91	77.56 ± 4.67	9.36 ± 6.94	70.31 ± 13.39
0.948	26.53 ± 0.32	76.90 ± 7.69	7.78 ± 5.70	69.41 ± 16.34
0.920	22.30 ± 1.02	73.47 ± 5.92	7.32 ± 5.46	70.91 ± 14.68

further evidence that DDH_2O-pretreated crescents maintained substantial membrane integrity even at $a_w = 0.920$.

Effects of Rehydration on Membrane Integrity

Rahman et al. (1976) indicated that damage to cell membranes may occur during rehydration. In order to test this, the concentration of leachate components was measured in the rehydration solution. The results of this evaluation are in Figures 15-20. Leaching of amino acids, conductive components and minerals was essentially negligible from the ethanol-pretreated crescents regardless of the dehydration a_w. These results were expected because the majority of the solutes were leached during pretreatment and dehydration. A summary of leaching from pretreatment through rehydration is given for amino acids and conductive components (Figures 21 and 22).

Unlike ethanol-pretreated crescents, crescents pretreated in DDH_2O retained relatively high concentrations of solute. Losses of these solutes to the rehydration bathing-solution increased sharply when crescents were dehydrated to a_w's of 0.987 or lower (Figures 15-20). Concentration of leachates in these solutions was statistically greater than 0.0 when crescents were dehydrated at a_w's of 0.980 or lower for Na, Mg, K and amino acids and at 0.970 or lower for conductivity and Ca. Although no change in membrane integrity was detected during dehydration of DDH_2O-pretreated crescents, the dehydrated crescents appear to have been predisposed to rehydration damage.

Figure 15. Effect of pretreatment and a_w of dehydration solution on leaching of amino acids in the rehydration solution. Bars indicate standard error of the mean for 3 replicates.

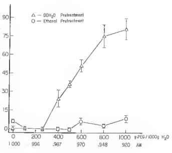

△ — DDH$_2$O Pretreatment
○ — Ethanol Pretreatment
Amino Acids (μmoles/g Dry Celery)
90
75
60
45
30
15
0
0
200
400
600
800
1000
g PEG/1000g H$_2$O
1.000
.996
.987
.970
.948
.920
AW

Figure 15. Effect of pretreatment and a_w of dehydration solution on leaching of conductive components in the rehydration solution. Bars indicate standard error of the mean for 3 replicates.

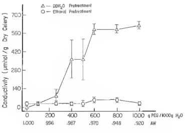

△ — DDH$_2$O Pretreatment
○ — Ethanol Pretreatment
Conductivity (μmhol /g Dry Celery)
700
560
420
280
140
0
0
200
400
600
800
1000
g PEG /1000g H$_2$O
1.000
.996
.987
.970
.948
.920
AW

Figure 17. Effect of pretreatment and a_w of dehydration solution on leaching of calcium in the rehydration solution. Bars indicate standard error of the mean for 3 replicates.

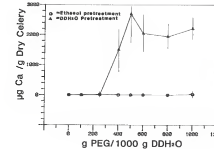

○ =Ethanol pretreatment
▲ =DDH₂O Pretreatment
µg Ca /g Dry Celery
3000
2000
1000
0
0
200
400
600
800
1000
1200
g PEG/1000 g DDH₂O

Figure 18. Effect of pretreatment and a_w of dehydration solution on leaching of sodium in the rehydration solution. Bars indicate standard error of the mean for 3 replicates.

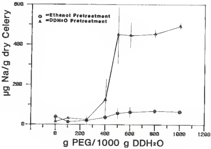

○ =Ethanol Pretreatment
▲ =DDH₂O Pretreatment
µg Na/g dry Celery
600
400
200
0
0
200
400
600
800
1000
1200
g PEG/1000 g DDH₂O

Figure 19. Effect of pretreatment and a_w of dehydration solution on leaching of magnesium in the rehydration solution. Bars indicate standard error of the mean for 3 replicates.

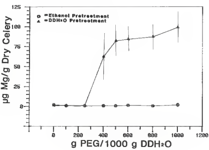

○ =Ethanol Pretreatment
▲ =DDH₂O Pretreatment
μg Mg/g Dry Celery
125
100
75
50
25
0
0
200
400
600
800
1000
1200
g PEG/1000 g DDH₂O

Figure 20. Effect of pretreatment and a_w of dehydration solution on leaching of potassium in the rehydration solution. Bars indicate standard error of the mean for 3 replicates.

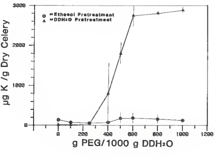

o =Ethanol Pretreatment
▲ =DDH₂O Pretreatment
µg K /g Dry Celery
3000
2000
1000
0
0
200
400
600
800
1000
1200
g PEG/1000 g DDH₂O

Figure 21. Cumulative losses of conductive components during pretreatment, dehydration and rehydration.

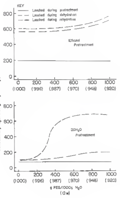
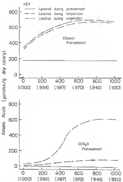

KEY
Leached during pretreatment
Leached during dehydration
Leached during rehydration
800
600
400
200
0
0
(1000)
200
(996)
400
(987)
600
(970)
800
(948)
1000
(920)
Ethanol Pretreatment
Conductivity (μmho/g dry celery)
800
600
400
200
0
DDH₂O Pretreatment
0
(1000)
200
(996)
400
(987)
600
(970)
800
(948)
1000
(920)
g PEG/1000g H₂O
(Ow)

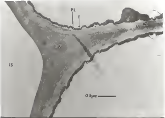
Figure 22. Cumulative losses of amino acids during pretreatment, dehydration and rehydration.

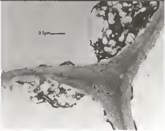

KEY
Leached during pretreatment
Leached during dehydration
Leached during rehydration
800
600
400
200
0
Ethanol Pretreatment
0
200
400
600
800
1000
(1,000)
(996)
(987)
(970)
(948)
(920)
Amino Acids (µmoles/g dry celery)
800
600
400
200
0
DDH$_2$O Pretreatment
0
200
400
600
800
1000
(1000)
(996)
(987)
(970)
(946)
(920)
g PEG/1000g H$_2$O
(Ωw)

When membrane integrity was not altered during pretreatment, crescents increased in weight at a_w's of 1.000 and 0.999. Ethanol-pretreated crescents, however, never regained weight lost in the pretreatment, even when placed in an a_w of 1.000. These data indicate that an intact membrane is essential for maintenance and recovery of fresh weight.

Comparisons of rehydrated tissue and tissue maintained in DDH_2O revealed extensive damage to the tonoplast and plasma membrane, whereas the tissue maintained in DDH_2O contained cells with intact plasma and tonoplast membranes (Figure 23). There was no apparent difference between ethanol-pretreated cells and cells from rehydrated tissue, suggesting that ethanol pretreatment and rehydration have similar effects on ultrastructure. The walls from the tissues described were indistinguishable from those of fresh tissue. All of the walls observed stained evenly, were rigid in appearance, and maintained a normal cell shape. It is difficult to distinguish between the effect of rehydration on the tonoplast and on the plasma membrane. In this study it was oberved that the thickness and the perimeter appeared to be quite similar, so that if the minimum surface-area of membranes is a critical parameter for membrane damage, the tonoplast would be expected to behave in a manner similar to the plasma membrane. The TEM observations may reflect the actual state of the membranes, however, some changes may have occurred during fixation. Some of the membrane lipid may have been washed away in the ethanol and acetone dehydration series. Membrane structures may also have been altered prior to crosslinking with OsO_4. Vacuum damage may

Figure 23. Effect of rehydration on ultrastructure of celery parenchyma cells. Cell wall junction of celery tissue not dehydrated (above) and dehydrated to 0.920 a_w then rehydrated (below). c = cytoplasm, PL = plasmalemma, T = tonoplast, CW = cell wall, IS = intercellular space, and ML = middle lamella.


PL
T
Mi
CW
IS
0 5μm


0 5μm
C
CW

occur during fixation, it is possible that the damage observed in TEM does not accurately reflect the effects of rehydration damage.

Physical Changes Resulting from Dehydration and Rehydration

The loss of membrane integrity during rehydration had an almost identical effect on rigidity and piece height as ethanol pretreatment did (Figures 24 and 25). When the membranes maintained integrity, the modulus of elasticity was high (about 5.5 MPa). However, when crescents were pretreated with ethanol or dehydrated to a_w's of 0.987 or lower, the rigidity was substantially lower (about 1.4 MPa). Because the decrease in modulus of elasticity occurred at the same a_w as the increase in leachate concentration, the decrease in piece height and at the a_w where the drop in rehydration ratio occurred, it is likely that intact cell membranes were responsible for about 4.5 MPa of the modulus of elasticity. Piece heights of crescents pretreated in DDH_2O and not dehydrated increased as a result of DDH_2O pretreatment. The mean height for those crescents was 0.59 cm as compared with the original 0.50 cm. The increase was dependent upon intact membranes. There was a sharp decrease in piece height at 0.987 a_w and when ethanol pretreatment was used.

Because crescents with impaired membranes exhibited some modulus of elasticity and heights close to their original height, other structures probably contribute to celery rigidity and structure. Increasing PEG concentrations from 400-1000 g/1000 g DDH_2O had no effect on rigidity, so it is unlikely that PEG had a measurable effect on the cell wall. In TEM studies, cells from crescents pretreated with ethanol and those from rehydrated crescents showed no indication

Figure 24. Effect of pretreatment and a_w of dehydration solution on modulus of elasticity. Bars indicate t-intervals for 17-20 replications.

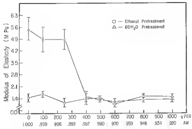
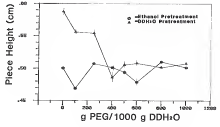

63
5.6
4.9
4.2
3.5
2.8
2.1
1.4
0.0
Modulus of Elasticity (M Pa)
○ — Ethanol Pretreatment
△ — DDH$_2$O Pretreatment
0
100
200
300
400
500
600
700
800
900
1000
g PEG / 1000g H$_2$O
1.000
.999
.996
.993
.987
.980
.970
.959
.946
.934
.920
AW

Figure 25. Effect of pretreatment and a_w on piece height. Bars indicate standard errors of the mean for 3 replicates.


Piece Height (cm)
.60
.55
.50
.45
g PEG/1000 g DDH₂O
0
200
400
600
800
1000
1200
● =Ethanol Pretreatment
▲ =DDH₂O Pretreatment

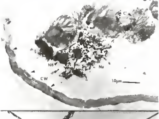
of cell wall or microfibrillar distortion or loss of middle lamella integrity that might be indicative of increased crystallinity. However, TEM observations of hot-air dried celery revealed many cell wall changes, including collapse and distortion of the cell walls, clumping of microfibrils, uneven thickness of cell walls, and splitting of the middle lamellae (Figures 26 and 27). These changes likely represent increased crystallinity. The initial portion of the force deformation curves for both hot-air dried and freeze dried crescents was flat and was regarded as 0.0 MPa. The difference between a rigidity of 0.0 MPa and one of 1.4 MPa appears to be the contribution of intact cell walls and middle lamellae. Because of the small, however significant, difference of piece height between treatments, it may be concluded that cell wall integrity has a greater effect on piece height than does membrane integrity.

Mechanism of Textural Changes

In the literature review section several possible mechanisms of dehydration damage were presented. Steponkus et al. (1981) proposed that dehydration damage is the result of a series of lethal stresses. Many of the stresses may not effect texture or otherwise affect quality.

Meryman (1968) indicated that, at a_w's of 0.973 or lower, red blood cells in NaCl became leaky to the bathing solution and therefore would not hypertonically contract beyond a "minimum volume." He proposed that damage to the red blood cells was incited by the pressure differential which occurs at that a_w. The data on celery provide evidence that cells of the celery petiole did not become leaky

Figure 26. Effect of dehydration to 6% moisture on shape and size of celery parenchyma cells. High magnification (above) and lower magnification (below), NB = nuclear bodies, Nu = disrupted nuclear material, CW = cell wall, C = cytoplasm arrows indicate clumped cell wall material.


C
NB
Nu
CW
1.0µm

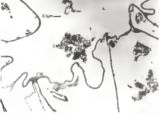
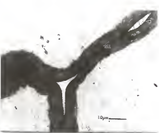
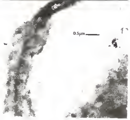

0.5µm
C
CW

Figure 27. Effect of dehydration to 6% moisture on the orientation of microfibrils and middle lamellae of celery parenchyma cells. Arrow indicates splitting of middle lamella (ML) (above) and portions of the wall are otherwise distorted. Microfibrils appear to clump and walls are irregular in thickness (below).


1.0µm


0.5µm

at a_w's of 0.973 or lower. This study employed a lower range of a_w's than did Meryman and over this range there appeared to be no impediment to weight loss (which would result in volume reduction) (Table 1). Dehydration leachates for crescents pretreated in DDH_2O gave no indication of leakiness. Another indication that cell membranes of DDH_2O-pretreated tissue maintained differential permeability is that at every a_w ethanol pretreated crescents exhibited lower dehydration weight retentions. In Meryman's experiments, the increase uptake of NaCl and the concomittant loss of K could have been a result of NaCl induced lesions (Jensen and Dettmeier, 1984).

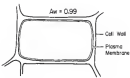
Other mechanisms of dehydration damage appear to involve stresses which occur at much lower water contents or a_w's. Lipid oxidation and formation of free fatty acids may promote a gelling or phase change in membranes (Senaratna et al., 1984), but are only important at very low a_w's (Bonzas and Karel, 1984). Likewise membrane changes from lamellar to HII do not occur until tissue has been dried below 20%. This mechanism does not explain damage observed at moisture contents above 50%.

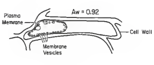
In agreement with Sterling and Shimazu (1961) cell wall and middle lamella changes (increased crystallinity) had a very significant effect on texture and rehydratability of celery crescents dried to very low moisture contents. At these low moisture contents oxidation of membrane lipids and membrane phase changes are additional limiting factors. No changes in the cell wall and middle lamella at

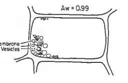
a_w's of 0.920-1.000 were observed and if changes occurred they had a negligible influence on texture.

Of the mechanisms reviewed by the author, the results are most characteristic of the expansion-induced lysis mechanism of membrane damage (Wiest and Steponkus, 1978). According to this mechanism, portions of the plasma membrane pinch off during hypertonic contraction when the membrane cannot be further compressed. Consistent with the authors' findings and differing from the mechanism of Meryman (1968), cell membranes of the contracted cells would not necessarily lose differential permeability (Figures 9-14, DDH_2O pretreatment). In a later study Steponkus et al. (1983) demonstrated with TEM and SEM micrographs of rye protoplasts that the pinched-off portions are endocytotic and are unable to recombine with the membrane proper when the dehydrated cell is placed in hypotonic conditions. The membrane bursts because its decreased surface area cannot accommodate the expanding volume. Surface area of celery protoplasts was not measured in this study, so a critical surface area could not be determined. However, since PEG is excluded by the tissue (Carpita et al., 1979), the lowering of a_w would result in a decreased protoplasmic volume and possibly a reduced surface area. Ranges for the minimum a_w and weight retention were determined. The application of this mechanism to controlled reduction of water activity in celery crescents is illustrated in Figure 28. Prior to dehydration the plasma membrane is closely appressed to the cell wall (for illustration purposes the plasma membrane is shown slightly pulled away from the wall). When the tissue is dehydrated to

Figure 26. Proposed mechanism of membrane changes occurring during dehydration and rehydration and resulting in loss of fresh texture. Cell from fresh tissue (above) after dehydration (center) and rehydration (below). Cell organelles, etc. were omitted for simplicity.


Aw = 0.99
Cell Wall
Plasma
Membrane


Aw = 0.92
Plasma
Memrane
Cell Wall
Membrane
Vesicles


Aw = 0.99
Membrane
Vesicles

tissue is dehydrated to a_w's of 0.920, plasmolysis occurs concomitant with a collapse of the cell wall. At 0.920 a_w, the cell wall is not detectibly compromised, because when the tissue is rehydrated at 1.000 the cell wall recovers. The damage the plasma membrane incurred at a_w's of 0.987 or lower, however, is not reversible. Using the mechanism of Wolfe and Steponkus (1979), portions of the plasma membrane, that have formed vesicles endocytotically, do not recombine with the membrane and the decreased surface area of the membrane causes it to lyse as the protoplast takes up water. If the membrane was totally destroyed at a_w's of 0.987 or lower, a complete equilibration of the cell contents with the bathing solution would be expected. However, there was an increase in leaching as a_w decreased from 0.987 to 0.920, indicating that some of the cell solutes still remained at the intermediate a_w's. This behavior may be explained by the observations of Morris and McGrath (1981) who reported that the vesicles they made resealed after expansion-induced lysis and retained osmotic responsiveness. Likewise if vesicles formed in the celery parenchyma cells after the plasma membrane lysed, the vesicles would likely contain solutes at a higher concentration than the bathing solution. These vesicles would also burst as water entered in, resealing, etc., until an equilibrium of water potentials was achieved across the vesicle membranes.

It appears that the primary stress affecting membrane permeability, and therefore rigidity, is a result of dehydration of the protoplast to a_w's of 0.987 or lower. As was shown earlier, dehydration itself, to a_w's of 0.920-1.000, does not impair differential permeability of the membrane, but predisposes the membrane to damage during hypotonic expansion.

Rahman et al. (1976) produced an intermediate moisture celery with increased rigidity and acceptability. The modulus of elasticity for the rehydrated intermediate moisture product was 21 Kg/cm^2 or about 1/3 of 60 Kg/cm^2, the modulus of elasticity for the fresh product. The highest yield was about 67% of fresh weight. In this study even the most severely treated crescents achieved approximately 1/3 of their original fresh rigidity and 70-80% of their fresh weight. Since this intermediate moisture celery had similar properties to rehydrated crescents in this study, it is highly likely that they produced a celery with intact cell walls and middle lamellae, but devoid of sound plasma and tonoplast membranes and that the same mechanism of membrane damage is involved.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

Conclusions

Insight into the relationship of ultrastructure to biophysical properties and especially texture has been gained. The following statements summarize these gains:

1. At a_w's above 0.987, the crescents were capable of complete rehydration. At a_w's below 0.987, a sharp increase in leaching of all measured components and a sharp decrease in modulus of elasticity occurred. Since cell walls and middle lamellae of all osmotically dried crescents were indistinguishable from cell walls in fresh tissue, this decrease in modulus of elasticity is likely the result of ruptured cell membranes. Expansion-induced lysis appears to be the primary stress responsible for the inability of celery to regain fresh rigidity.

2. Cell wall and middle lamella changes were not observed at a_w's of 0.920-1.000. At moisture contents of about 6%, a change in microfibril spacing, an increase in cell wall distortion, irregular thickness and a splitting of the middle lamella were observed. These changes may be indicative of an increase in cell wall crystallinity. When cell walls and middle lamellae were in this condition, crescents exhibited no rigidity. When cell walls and middle lamellae were intact and cell membrane integrity was disrupted, crescents exhibited

1/3 of the rigidity of fresh crescents. Approximately 1/3 of the rigidity of fresh tissue can be attributed to the cell walls and middle lamellae. The remaining 2/3 are the effect of intact plasma and tonoplast membranes pressing against firmly planted cell walls.

3. The approach used in this study to distinguish between membrane and cell wall effects could be used for a variety of fruit and vegetable studies. A comparison of leachate conductivity and amino acids was a good indicator of membrane integrity. Modulus of elasticity taken at the initial portion of the force deformation curve was a useful index of rigidity.

4. Polyethylene glycol, which is used frequently in the plant sciences for water potential studies, could be used to prepare fruits and vegetables to known a_w's ranging from about 0.920 and upward. Under constant agitation, equilibration is more rapid compared with equilibration over saturated salts. In this way, problems with microbial growth can be avoided.

5. A model based on the above observations and those of Steponkus et al. (1981) is proposed. It is suggested that as the a_w is lowered beyond 0.987 the plasma membrane is unable to further compress and pinches off endocytotically. The reduced outer surface of the membrane prevents it from expanding to the original size and the membrane bursts in hypotonic conditions. The cell wall did not appear to be permanently affected by PEG.

Suggestions for Future Work

The lethal stresses or irreversible changes which occur when susceptible tissue such as celery petioles are dehydrated to low moisture contents are many and complex. These problems could be entirely circumvented by preparing an intermediate moisture product. The a_w required for microbial stability is less than 0.7. Rahman et al. (1976) prepared intermediate moisture celery with physical properties very like the celery which was dehydrated to 0.920 (regardless of the pretreatment). It does not appear that the intermediate moisture tissue underwent any additional stress by the addition of a_w-reducing solutes. This needs to be verified.

If the only stress incurred at intermediate moistures is that of predisposition of the membrane to expansion-induced lysis, then a focus could be placed on the membrane and ways to avoid pinching off of vesicles which occurs when the surface area is reduced. Three possible approaches for avoiding expansion-induced lysis are (1) acclimation, (2) plant selection, and (3) crosslinking agents.

1. Steponkus et al. (1983) indicated that protoplasts previously acclimated to freezing conditions had an increased ability to expand and remain viable after hypertonic contraction. This investigatory approach could include the use of an imposed stress, either cold or low moisture stress, while the plants were under growing conditions. This tissue could be harvested and subjected to the type of experiments done in this study.

2. There is a rich historical basis for plant selection (Poehlman, 1959). Tomatoes have been selected for size, shape,

solids, etc. for convenience in processing. It is possible that celery could be selected for an increased resistance to expansion-induced lysis.

3. Crosslinking has been used successfully to preserve membranes in electron microscopy (Hayat, 1981). Prior to dehydration, a pretreatment with glutaraldehyde, a crosslinker of proteins, in Karnovky's (1965) fixative followed by osmium tetroxide, a crosslinker of lipids, could be employed to strengthen the link of membrane vesicles with the plasma membrane and reincorporation into the plasma membrane during hypotonic expansion.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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